REMARKS

Upon entry of this amendment, claims 15, 21-24, 31, and 62-79 are pending. Claims 15, 21-24, 31, 63, 71, and 72 are amended. Claims 1-14, 16-20, 25, 26, 27, 28, 29, 30, and 32-61 are cancelled or were previously cancelled. Claims 74-79 are newly added. The claim amendments are discussed in detail below.

Request for Continued Examination

This letter is being submitted along with a Request for Continued Examination (RCE) pursuant to 37 CFR 1.114. An Information Disclosure Statement (IDS) is also being submitted herewith.

Amendments to the Claims

Claim 15 is amended herein. As amended, the claim recites a method of reducing solid tumor volume in an animal or human in need thereof, the method comprising *inhibiting γ-secretase activity and inhibiting angiogenesis* in the tumor, independent of Notch cleavage, to reduce solid tumor volume in the animal or human. Support for these amendments can be found in the specification, for example, in paragraph [0063].[1] Other claims are amended in accordance with the amendments to claim 15.

Rejection under 35 U.S.C. §112, first paragraph

Reconsideration is requested of the rejection of claim(s) 15, 21-24, 31, and 62-73 under 35 U.S.C. §112, first paragraph, on the asserted basis that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors were in possession of the claimed invention at the time the application was filed.

[1] Paragraph numbers cited throughout this response refer to the applicants' published patent application, US2004/0229816.

According to the Office, applicants' claims are of impermissibly broad scope because the term "secretase inhibitor" is a very broad generic statement drawn to any secretase inhibitor, and there exists a plethora of such compounds, which are not adequately described and/or represented in the examples. This assertion, however, is generally inconsistent with the applicants' specification. Several examples of secretase inhibitors, including γ-secretase and β-secretase inhibitors, useful in the applicants' methods are disclosed in the specification. In particular, the aspartyl protease transition-state γ-secretase inhibitor L-685,458; the dipeptide protease γ-secretase inhibitors DAPT and DAPM; the isocoumarin-based serine protease γ-secretase inhibitor JLK-6; the substrate analogue peptide β-secretase inhibitors Z-VLL-CHO and GLI89; and the peptidomimetic tight binding transition-state analogue β-secretase inhibitor OM99-2 are specifically disclosed in the specification, and are tested in the working examples. Thus, applicants' specification, including the disclosure of several representative secretase inhibitors reasonably conveys to one skilled in the art that the applicants were in possession of the methods for reducing solid tumor volume, as claimed, at the time of filing the application.

In order to advance prosecution, claim 15 has been amended to require the inhibition of γ-secretase activity and angiogenesis **in concert**, to reduce solid tumor volume. Importantly, the claim also requires that said inhibition is ***independent of Notch cleavage***. As pointed out in the applicants' specification, the γ-secretase inhibitor JLK-6, for example, reduces angiogenesis in vitro and inhibits the growth of human lung xenografts in nude mice.[2] Moreover, this γ-secretase inhibitor does not affect Notch processing.[3] Therefore the anti-angiogenic activity has been shown to be independent of Notch cleavage. For these and other reasons, in both human brain and human lung tumor models the anti-tumor activity of the γ-secretase inhibitors is

[2] See applicants' specification, paragraphs [0063], [0081]-[0084].
[3] See, e.g., Petit et al., Nature Cell Biotechnology 3, 2001, 507-511; Petit et al., J. Neuroscience Res. 74, 2003, 370-377.

mediated by the inhibition of angiogenesis, because microvessel density values in the treated tumors have been shown to be significantly decreased.[4]

As detailed in applicants' Example 1, for instance, γ-secretase inhibitors L-685,458, DAPT, DAPM, and JLK-6 have a potent angiogenic effect on capillary morphogenesis, suggesting that γ-secretase activity is required during the angiogenesis process.[5] Further supporting the involvement of a γ-secretase-like activity during angiogenesis, Example 2 and corresponding data shows that two γ-secretase inhibitors, DAPT and L-685,458, inhibited the sprouting of microvessels from explants of rat aortae.[6] And, Example 3 and corresponding data shows that the γ-secretase inhibitor DAPT not only completely inhibited the growth of U-87 MG brain tumors, but also reduced the volume of the tumors by **more than 90%** after one week of treatment.[7] A decreased vascularization was also observed in U-87 MG tumors treated with DAPT compared with the control, suggesting that DAPT was able to inhibit tumor angiogenesis in vivo. DAPT also potently suppressed the growth of A-549 lung adenocarcinoma tumors in nude mice, and vascularization appeared to be decreased following DAPT treatment, suggesting in vivo inhibition of angiogenesis.[8] Another γ-secretase inhibitor, JLK-6, inhibited the growth and vascularization of human lung adenocarcinoma tumors xenotransplanted into nude mice.[9]

For at least the foregoing reasons, it is clear that the applicants were in possession of a method for reducing solid tumor volume by inhibiting γ-secretase activity and angiogenesis, independent of Notch cleavage. The claims directed to this method, therefore, satisfy 35 U.S.C. §112, first paragraph.

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[4] See applicants' specification, paragraph [0063].
[5] Applicants' specification, paragraphs [0065]-[0076], Figs. 1(b), 2(b), 3(c).
[6] Applicants' specification, paragraphs [0077]-[0080], Figs. 7(b), 7(c).
[7] Applicants' specification, paragraphs [0081]-[0084], Figs. 8(a), 8(c).
[8] Id., Figs. 9(a), 9(c).
[9] Id., Fig. 10.

Rejection under 35 U.S.C. §103(a)

Reconsideration is requested of the rejection of claims 15, 21-24, 31, 62-73 under 35 U.S.C. § 103(a) as being unpatentable over Weng et al. (Molecular and Cellular Biology 23(2), 2003, 655-664), in view of Jundt et al. (Blood 100(11), PG-Abstracts No. 594, 2002).

Weng et al. discloses the use of recombinant T-cell lines transformed to express Notch-constructs in connection with a study of Notch signaling in regulation of cell growth and differentiation. Weng et al. report that presenilin inhibitors alter the processing of Notch constructs *in vitro*, and that one specific inhibitor, DPF-AA, was able to inhibit the growth of one of the recombinantly created cell lines. Figure 2 shows the ***Notch-dependent*** inhibition of transcriptional stimulation in U20S cells using a range of presenilin inhibitors, including DAPT.

Jundt et al. discloses the characterization of Jagged1-Notch signaling in Notch-positive Hodgkin and large cell anaplastic lymphoma cell lines. In the lone inhibition study disclosed, Jundt et al. report that, *in vitro*, exposure of these cell lines to Jagged1 results in an exponential increase in the cells' respective growth rates, and this effect can be blocked using the γ-secretase inhibitor, DAPT. Significantly, Jundt et al. report that only the increase in growth rate caused by Jagged1 exposure is blocked by DAPT. In accordance with these findings, Jundt et al. state that ***interruption of Notch signaling*** by γ-secretase inhibitors might be a novel therapeutic approach to control neoplasm proliferation.

Weng et al. and Jundt et al. each emphasize the role of Notch inhibition in reducing cell proliferation. The experiments detailed in the cited references are also performed solely *in vitro*, and there is nothing to suggest that the compounds would have a similar effect *in vivo*. Importantly, while Weng et al. and Jundt et al. each utilize DAPT, a compound having γ-secretase inhibiting activity, these references are **silent** on the inhibition of angiogenesis and the reduction of solid tumor volume, irrespective of the compound employed.

In contrast to Weng et al. and Jundt et al., claim 15 requires the inhibition of γ-secretase activity ***and angiogenesis***, independent of Notch cleavage, to reduce solid tumor volume. The cited references merely disclose that DFP-AA, DAPT, or DAPT analogs reduced the rate of cell proliferation in the cell lines tested, and state that this effect is due to the blockage of Notch signaling. They say nothing about reducing solid tumor volume, and nothing about inhibiting both γ-secretase activity and angiogenesis independent of Notch activity. Instead, their sole focus is on the inhibition of Notch.

As opposed to merely reducing *cell proliferation* by virtue of a Notch-dependent pathway, the applicants' method is directed to reducing ***solid tumor volume*** by an entirely different mechanism. Notably, in the claimed method for reducing solid tumor volume, both γ-secretase activity and angiogenesis are inhibited, and such inhibition is independent of Notch processing.

Furthermore, and with all due respect, third party publications not cited by the Office are inconsistent with the Office's postion.

Koch et al. (Cell. Mol. Life Sci. 64, 2007, 2746-2762) and Dotto (Oncogene 27(38), 2008, 5115-5123), for example, report that Notch1-**deficient** animals develop spontaneous, highly vascularized tumors.[10] Using the logic of the Office, Notch deficiency should prevent such tumor growth and vascularization.

Leong et al. (Molecular and Cellular Biology 22(8), 20020, 2830-2841) report that Notch4 (one of four Notch homologues) inhibition in human mammary epithelial (HMEC) cells and avian Q2bn packaging cells actually *increases* angiogenesis. As seen in Fig. 1 of Leong et al., for example, reduced endothelial cell sprouting is demonstrated when HMEC cells overexpress Notch4; thus, constitutively-activated Notch4 is anti-angiogenic.[11] Fig. 2 of Leong et al. is a particularly vivid demonstration of this *in vivo*. There, VEGF, which drives angiogenesis, is inhibited in its action in the presence of

[10] Koch et al., page 2753; Dotto, 5117-5118.
[11] Leong et al., page 2833.

Notch4-active Q2bn cells.[12] Inhibition of Notch4 in these cells, therefore, would increase angiogenesis, not decrease it.

Koch et al. also report only correlative evidence for the involvement of Notch signaling in human breast cancer.[13] In fact, they report that one-third of breast cancer samples do not even express Notch.[14] Clearly, therefore, breast cancers which do not express Notch would not be expected to be responsive to a Notch inhibitor.

When considered in the context of other published literature, Weng et al. and Jundt et al. provide a skilled artisan with no basis to predict that tumor volume may be reduced and angiogenesis and γ-secretase activity can be inhibited independent of Notch cleavage. Weng et al. and Jundt et al. merely disclose that DAPT induced Notch inhibition leads to a reduction in cell proliferation *in vitro*. In contrast, applicants have shown that compounds that have **no effect** on Notch can still inhibit γ-secretase activity and angiogenesis *in vitro* and *in vivo*.[15] Moreover, even if you do inhibit Notch, you will not necessarily inhibit angiogenesis, and instead may actually *increase* angiogenesis, as shown by Koch et al., Dotto, and Leong et al. Therefore, there is ***no way*** to predict that inhibition of angiogenesis and reduction in solid tumor volume will occur independent of Notch mediation. As reported by the applicants in paragraph [0063] and elsewhere in the specification, however, this is the case. The present disclosure that γ-secretase activity and angiogenesis can be inhibited to reduce solid tumor volume, independent of Notch cleavage, is therefore an unexpected and surprising finding.

For at least the reasons discussed above, claim 15 is patentable over Weng et al. alone or in combination with Jundt et al. Claims 21-24, 28, 30, 31, and 62-76 depend directly or indirectly from claim 15, and are patentable for the same reasons as claim 15 and by virtue of the additional elements they require. New claim 76, for instance, requires that γ-secretase activity and angiogenesis are inhibited without Notch cleavage, and Weng et al. and Jundt et al. teach away from this aspect. By way of

[12] Id., page 2834.
[13] Koch et al., page 2748.
[14] Id.
[15] Applicants' specification, paragraphs [0065]-[0076] and [0081]-[0084], and Figs. 1(b), 2(b), 3(c), and 10.

another example, new claim 78 recites that the γ-secretase inhibitor is JLK-6, and this inhibitor does not affect Notch processing.

CONCLUSION

In view of the foregoing, favorable consideration and allowance of claims 15, 21-24, 31, and 62-79 is requested. The Examiner is invited to contact the undersigned should any issue remain unresolved. The Commissioner is hereby authorized to charge to Deposit Account No. 02-4467 any fees under CFR 1.16 and 1.17 which may be required during the pendency of this application.

Respectfully submitted,

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